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Review article

Lipids and lipoxidation in human brain aging. Mitochondrial ATP-synthase as a key lipoxidation target

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ABSTRACT

The human brain is a target of the aging process like other cell systems of the human body. Specific regions of the human brain exhibit differential vulnerabilities to the aging process. Yet the underlying mechanisms that sustain the preservation or deterioration of neurons and cerebral functions are unknown. In this review, we focus attention on the role of lipids and the importance of the cross-regionally different vulnerabilities in human brain aging. In particular, we first consider a brief approach to the lipidomics of human brain, the relationship between lipids and lipoxidative damage, the role of lipids in human brain aging, and the specific targets of lipoxidative damage in human brain and during aging. It is proposed that the restricted set of modified proteins and the functional categories involved may be considered putative collaborative factors contributing to neuronal aging, and that mitochondrial ATP synthase is a key lipoxidative target in human brain aging.

1. Lipidomics of human brain

The natural history of lipids is related to the origin of life and evolution of multicellularity [1]. The result is that all living organisms and cell types have lipid membranes. Neurons and glial cells are not an exception. Indeed, lipids supported and allowed the brain's evolution toward complexity [2,3].

Among tissues of the human body, nervous tissue is one of the richest in lipid content, representing 10–12% of the fresh weight and 50% of the dry matter [4]. In addition to their quantitative relevance, lipids of the nervous system show great structural and functional diversity, with most lipid categories represented in neural cells as expressions of the different needs and functions ascribed to them which are related to the generation of membranes, cell signaling, and energy storage [5,6].

A wide spectrum of functions, from motor to cognitive, are performed by human brain thanks to the organization and activity of a diversity of neural cells in a multitude of neuroanatomically and functionally different regions. It has been proposed that this diversity among neural cells, and particularly neurons, is phenotypically achieved, at least in part, through the expression of particular lipid profiles [6]. This region-dependent lipidomic fingerprint can play a key role in the differing vulnerability to aging exhibited by specific regions of the human brain [6–8].

1.1. Lipids in whole human brain

Whole adult human brain contains a large amount and diversity of lipid classes and lipid molecular species. Thus, in addition to the presence of diverse phospholipid classes, whole brain also contains a large portion of sphingolipid species, as well as a great pool of cholesterol and cholesterol metabolites [4] (see Fig. 1). Specifically, glycerophospholipids (Gpl) represent about 4.5–5.4% of wet weight (4.2% in the gray matter and 7% in the white). Among Gpl, ethanolamine glycerophospholipids (EtnGpl) are numerically the main phospholipid (35.6%) and most of these are ethanolamine plasmalogen (PE(P-); 50–60%). The fatty acid profile of this lipid class indicates a large content of polyunsaturated fatty acids (PUFAs), preferentially at sn-2 position [9,10]. The predominant form of choline glycerophospholipids (ChoGpl) is phosphatidylcholine (PtdCho or PC; 32.8%), with PC(16:0/

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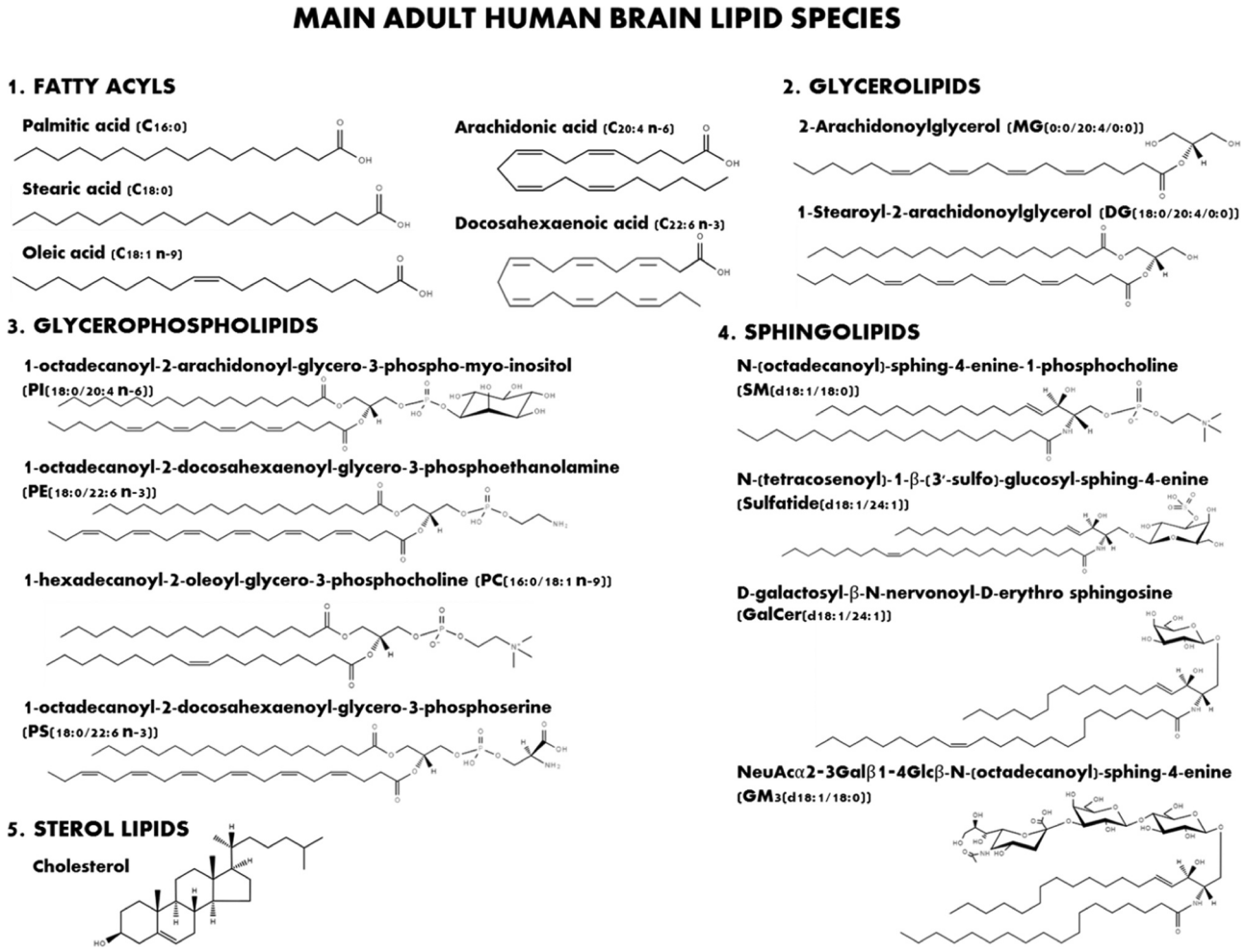

Fig. 1. Main adult human brain lipids: (1) major fatty acids present in adult human brain amounting to about 80–90% of total fatty acid profile [19]; (2) glycerolipids (GL) are fatty acid esters of glycerol and in human brain mostly comprise mono- and diacylglycerols; (3) glycerophospholipids includes distinct lipid classes based on the nature of the head group linked to the phosphate at the sn-3 position of the glycerol backbone: Phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositols (PI) are the main phospholipids present in human brain [4]; (4) representative sphingolipids present in human brain are shown; and (5) cholesterol is the main sterol lipid presents in human brain. Lipid molecular species structures are adapted from LipidMaps (https://www.lipidmaps.org/).

18:1) being the major molecular species [9,11]. The choline plasmalogen (PC(P-))and the alkyl (PC(O-)) analogue account for only about 2% of total ChoGpl. The concentration of serine glycerophospholipids (SerGpl) is about 16.6% of total phospholipids. They occur as phosphatidylserine (PtdSer or PS; more than 90%) and serine plasmalogen (PS(P-)). Regarding the fatty acid profile, they contain mainly stearic acid (18:0), oleic acid (18:1n-9), and docosahexaenoic acid (DHA, 22:6n-3) [9,10]. Gray matter PSs are particularly rich in DHA. Inositol glycerophospholipids (InoGpl) account for about 2.6% of the total phospholipids. Phosphatidylinositol (PtdIno or PI) and triphosphoinositide (PIP3) are the major lipids with only trace amounts of diphosphoinositide (PIP2), with the main fatty acid components 18:0 and arachidonic acid (AA, 20:4n-6) [4,11]. Glycerophosphates (Gpl), and specifically phosphatidic acid (PA), occur in low concentrations in brain (about 2% of total phospholipids). Finally, diphosphatidylglycerol (cardiolipin), mainly located in brain mitochondria, represents around 0.2% of phospholipids. The main fatty acids included in this phospholipid fraction are palmitic acid (16:0), palmitoleic acid (16:1), 18:0, 18:1n-9, and linoleic acid (18:2n-6), with minor representation, if present, of linolenic acid (18:3n-3) and AA [4,12].

Sphingolipids are a group of complex lipids present in particularly large concentrations in human brain. This group of lipids consists of sphingomyelin, cerebrosides, sulfatides, and gangliosides. Sphingomyelin (SM) accounts for about 14.8% of sphingolipid content. SM fatty acids are made up mainly of 18:0, lignoceric (24:0), and nervonic (24:1) acids [11]. Cerebrosides amount to 15.8% of the total lipids, mostly as galactosylceramide, and the fatty acid components characteristically contain hydroxyl fatty acids (cerebronic (24 h:0) and hydroxynervonic (24 h:1)) which account for more than 50% of the total fatty acids [11]. Sulfatides are the only sulfosphingolipid present in brain, and they account for about 6.2% of total lipids of brain. The fatty acid composition of sulfatides is similar to that of cerebrosides [11]. Finally, gangliosides are particularly abundant in brain. Stearic acid (18:0) is the main fatty acid and forms over 80% of the total ganglioside fatty acid content [4].

1.2. Lipid distribution in human brain: a cross-regional comparative approach

The complexity of the adult human brain is reflected by its 900 neuroanatomically precise subdivisions [13]. Analogously to the finding that there are region-specific transcriptomic [13] and proteomic [14] fingerprints, it may be proposed that the lipidome of each brain structure or region is also different. In this line, some recent studies have used a lipidomic approach to analyze the lipid profile of human brain gray matter at the mitochondrial and microsomal levels in three

different regions of the human cerebral cortex: entorhinal cortex (EC), hippocampus (HP), and prefrontal cortex (PFC) [15–17]. The results show that, independently of the subcellular fraction and brain region, the three major phospholipid classes are, in this order: ChoGpl, EtnGpl, and SerGpl. Overall, ChoGpls comprise around 43–55% of total phospholipids (43% in HP; 50% EC; and 55% PFC), and the main molecular species are PC(16:0/18:1), PC(16:0/16:0), and PC(18:0/18:1). EtnGpls represent about 28–36% of total phospholipids (36% in HP; 32.1% EC; and 30% PFC), with the major molecular species (in this order): PE (18:0/22:6), PE(18:0/20:4), PE(18:0/22:4), and PE(16:0/22:6). Finally, SerGpls represent around 15–21% of total phospholipids (21% in HP; 18% EC; and 15% PFC), with the major molecular species (in this order): PS(18:0/22:6), and PS(18:0/18:1). Other relevant (and minor) phospholipids at the mitochondrial level like PI and cardiolipin were not analyzed. Currently, however, no additional information is available for a comprehensive atlas of the adult human brain lipidome.

From another approach, an interesting scenario can be drawn when cross-regional differences in fatty acid profiles are considered. Fatty acids are prime components of the structural diversity of lipids of any cell type, neural cells included. Indeed, with different permutations of head group and fatty acids up to 10,000 theoretical lipid molecular species can be generated [18]. Fatty acids also determine the functional properties of lipids in brain which can be ascribed to their roles in the structural and functional integrity of neuronal and glial cell membranes, the generation of lipid signaling mediators, and the chemical reactivity of the acyl chains [6]. In this context, a recent study analyzing the fatty acid composition of 12 differential regions of the adult human central nervous system (from spinal cord to cerebral cortex) [19] found shared traits such as an average chain length of 18 carbon atoms, and a relative distribution between saturated (SFA) and unsaturated (UFA) fatty acids of 40:60 (see Table 1).

When we focused on specific fatty acids, the findings showed that the most differential fatty acids are 18:1n-9, 20:1n-9, 20:4n-6, 22:4n-6, and 22:6n-3 [19] (see Table 1). Among these, the monounsaturated fatty acid (MUFA) 18:1n-9 is the most abundant (with a cross-regional range from 24% to 36%), whereas 22:6n-3 and 20:4n-6 are the most abundant PUFAs in all regions, ranging (together) from 5% to 16%. The finding that DHA (22:6n-3) is the main PUFA present in brain regions is in line with previous findings pointing up the very high level of DHA in the whole brain [4], but this is clearly in contrast to spinal cord and medulla oblongata where the content is the lowest [19]. Interestingly, it was observed that the greater the presence of MUFAs in a given region, the lower the PUFA content.

For specific cross-regional differences in DHA (22:6n-3) content, it is relevant to mention that these differences are due to variations in desaturase and peroxisomal beta-oxidation activity, enzymes involved in the PUFA biosynthesis pathway. And more interestingly, this activity can be specifically ascribed to neurons [19]. Consequently, the protein expression linked to PUFA biosynthesis supports the cross-regional differences in fatty acid profiles and suggests the possibility that neurons can actively maintain their own compositional profile.

2. Lipoxidative damage and human brain

2.1. Lipid oxidation and lipoxidation-derived molecular damage

Two traits of the membrane lipids determine their vulnerability to lipoxidative damage: the physico-chemical properties of the membrane

Table 1
Fatty acid profiles of 12 different regions from adult healthy human central nervous system.

Fatty Acid	SC	MO	CB	SN	TH	AM	ST	EC	HC	TC	OC	FC
14:0	1.9	1.8	1.1	1.3	1.3	1.2	1.0	0.8	1.3	1.0	1.2	1.3
16:0	18.2	18.2	25.5	20.2	20.5	24.3	21.6	21.8	22.1	23.4	23.7	20.0
16:1n−7	1.5	1.7	1.2	1.4	1.0	1.1	1.1	0.9	1.3	1.1	1.3	1.5
18:0	19.0	18.1	17.8	19.0	18.4	19.5	20.7	22.4	20.1	20.1	21.2	20.4
18:1n−9	36.2	36.7	23.9	32.1	29.5	24.9	25.7	28.0	27.0	24.3	24.8	24.6
18:2n−6	0.5	0.6	1.0	0.8	0.8	0.7	0.8	0.8	1.3	0.8	1.1	1.2
18:3n−3	0.2	0.3	0.2	0.2	0.3	0.1	0.4	0.1	0.1	0.2	0.1	0.2
18:4n−3	0.2	0.2	0.6	0.3	0.3	0.3	0.3	0.1	0.2	0.4	0.1	0.4
20:0	0.6	0.5	0.7	0.4	0.5	0.3	0.6	0.2	0.3	0.8	0.3	0.5
20:1n−9	6.3	4.6	1.2	2.5	2.2	0.9	1.6	1.2	1.3	1.1	1.3	1.7
20:2n−6	0.4	0.3	0.3	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.3	0.4
20:3n−6	1.4	1.4	1.5	1.3	1.4	1.2	1.1	1.0	1.2	1.4	1.4	1.1
20:4n−6	4.0	4.5	7.2	6.2	6.5	8.4	7.5	7.7	7.6	6.6	6.6	6.9
20:5n−3	0.3	0.2	0.4	0.4	0.5	0.3	0.4	0.1	0.3	0.4	0.3	0.4
22:0	0.6	0.6	0.5	0.6	0.6	0.3	0.5	0.2	0.5	0.5	0.3	0.5
22:4n−6	3.4	3.9	3.0	4.5	4.9	5.4	5.2	4.9	5.4	4.8	3.9	4.8
22:5n−6	0.3	0.4	1.1	0.5	0.8	0.9	0.9	0.8	1.1	0.9	0.7	1.0
22:5n−3	0.3	0.3	0.6	0.6	0.6	0.5	0.5	0.3	0.4	0.5	0.4	0.7
22:6n−3	1.7	2.2	10.0	5.0	6.8	7.5	7.1	7.1	6.6	9.3	9.4	10.4
24:0	1.4	2.1	0.7	0.8	1.0	0.7	0.9	0.4	0.7	0.8	0.4	0.9
24:5n−3	0.2	0.2	0.2	0.2	0.3	0.1	0.2	0.1	0.1	0.1	0.1	0.2
24:6n−3	0.4	0.4	0.3	0.3	0.4	0.2	0.4	0.04	0.1	0.2	0.2	0.1
ACL	18.1	18.2	18.3	18.2	18.4	18.3	18.4	18.3	18.3	18.4	18.3	18.5
SFA	41.8	41.5	46.6	42.5	42.5	46.5	45.6	45.9	45.2	46.8	47.3	43.7
UFA	58.1	58.4	53.4	57.4	57.4	53.4	54.3	54.0	54.7	53.1	52.6	56.2
MUFA	44.2	43.1	26.4	36.1	32.9	26.9	28.4	30.3	29.6	26.7	27.5	27.9
PUFA	13.9	15.3	26.9	21.3	24.5	26.4	25.9	23.7	25.1	26.4	25.0	28.2
PUFAn−3	3.6	4.0	12.5	7.4	9.4	9.2	9.7	8.0	8.1	11.4	10.8	12.6
PUFAn−6	10.3	11.2	14.3	13.9	15.0	17.2	16.1	15.7	16.9	15.0	14.2	15.6
DBI	101.6	107.2	152.7	130.2	143.5	146.5	145.3	137.4	140.9	149.6	144.2	160.0
PI	61.4	68.9	146.8	105.1	125.1	135.0	132.2	121.3	124.5	142.0	135.1	153.3

For fatty acids, values are means from 5 to 8 different adult healthy subjects and are expressed as mol%.
Fatty acid indexes: ACL, average chain length; SFA, saturated fatty acids; UFA, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA n-6 or n-3, polyunsaturated fatty acids n-3 or n-6; DBI, double bound index; PI, peroxidizability index. For calculations, see [19].
Abbreviations: SC, spinal Cord; MO, medulla oblongata; CB, cerebellum; SN, substantia nigra; TH, thalamus; AM, amygdala; ST, striatum; EC, entorhinal cortex; HC, hippocampus; TC, temporal cortex; OC, occipital cortex; FC, frontal cortex.
Adapted with permission from Naudi et al. [19].

bilayer and the chemical reactivity of the fatty acids that compose it [20,21]. The first property is based on the preferential solubility of reactive species (e.g., free radicals) in the fluid lipid bilayer instead of in aqueous solution [22,23]. The second and more relevant property is related to the fact that PUFA residues of lipids (and particularly Gpl) are very sensitive to oxidation, and this sensitivity increases as a function of the number of double bonds per fatty acid molecule [24–26]. So, PUFA side chains (with two or more double bonds) are much more easily damaged by reactive species than are MUFA (one double bond); while SFA (no double bonds) are resistant to peroxidation.

Lipid peroxidation generates hydroperoxides and endoperoxides, which undergo fragmentation to produce reactive intermediates with different carbons in length called reactive carbonyl species (RCS). The most reactive RCS are α,β-unsaturated aldehydes [4-hydroxy-2-nonenal (HNE) and acrolein], di-aldehydes [malondialdehyde (MDA) and glyoxal], and keto-aldehydes [4-oxo-2-nonenal (ONE) and isoketals] [27–30]. Special mention must be made of neuroketals (NKT) which are keto-aldehydes formed by the non-enzymatic oxidation of the 22:6n-3 through the neuroprostane pathway [31]. 2-Hydroxyheptanal and 4-hydroxyhexenal are other significant aldehydic products of lipid peroxidation of PUFAs. Additional reactive compounds are the cyclopentenone prostaglandins, levuglandins, or oxidized phospholipids, among others [32]. Overall, these RCS are ubiquitously generated and have two unique properties: i) a greater half-life compared with most reactive oxygen species (ROS); and ii) a non-charged structure that allows them to migrate easily through hydrophobic membranes and hydrophilic cytosolic media, thereby extending the migration distance far from the generation site [21].

RCS react with nucleophilic groups in macromolecules (lipoxidation reactions) like proteins [32,33], DNA [34], and aminophospholipids [35], resulting in their chemical, and non-enzymatic modification. The outcome is the generation of a diversity of adducts and intra- and inter-molecular cross-links collectively named Advanced Lipoxidation Endproducts (ALEs) [32,33,36] (see Fig. 2). Thus, by reacting with nucleophilic sites in proteins (belonging basically to Cys, Lys, Arg, and His residues), RCSs generate ALE adducts such as MDA-Lys, HNE-Lys, NKT-lys, FDP-Lys, carboxymethyl-lysine (CML), and S-carboxymethyl-cysteine, as well as the cross-links glyoxal-lys dimer (GOLD), and lys-MDA-lys, among others. The accumulation of MDA adducts on proteins is also involved in the formation of lipofuscin, a non-degradable intralysosomal fluorescent pigment formed through lipoxidation reactions already described in human neurons by Hannover in 1842 [37,38]. Most of these compounds have been detected, characterized, and located (by mass spectrometry, redox proteomics, and immunohistochemistry) in the human brain. RCSs can also react with the exocyclic amino groups of deoxyguanosine, deoxyadenosine, and deoxycytosine to form alkylated products. Guanine is the most prone and commonly modified DNA base because of its high nucleophilicity, and MDA-deoxyguanosine (M1dG) is the most common adduct. Finally, the amino group of aminophospholipids can also react with RCS, leading to the formation of adducts such as MDA-PtdEtn, and carboxymethyl-PtdEtn. The molecular consequences derived from ALE formation include protein alterations in physico-chemical properties (e.g., conformation, charge, hydrophobicity, and solubility), formation of intra- and inter-molecular protein crosslinks and aggregates, loss of enzymatic activity, DNA damage and mutagenesis, and alterations in physico-chemical and biological properties of the lipid bilayer [21,36].

Beside the cytotoxic effects, RCSs can also work as regulatory signals inducing adaptive responses specifically designed to decrease lipoxidative damage and improve antioxidant defenses [21,36,39]. Two of these mechanisms involved in the modulation of oxidative stress by RCS are: i) the modification and activation of uncoupling proteins (UCPs) by the RCS hydroxynonenal and the subsequent decrease in mitochondrial ROS production [40,41], and ii) the activation of the antioxidant response signaling pathway (Nrf2 pathway) that includes, among others, the expression of enzymes such as glutathione-S-

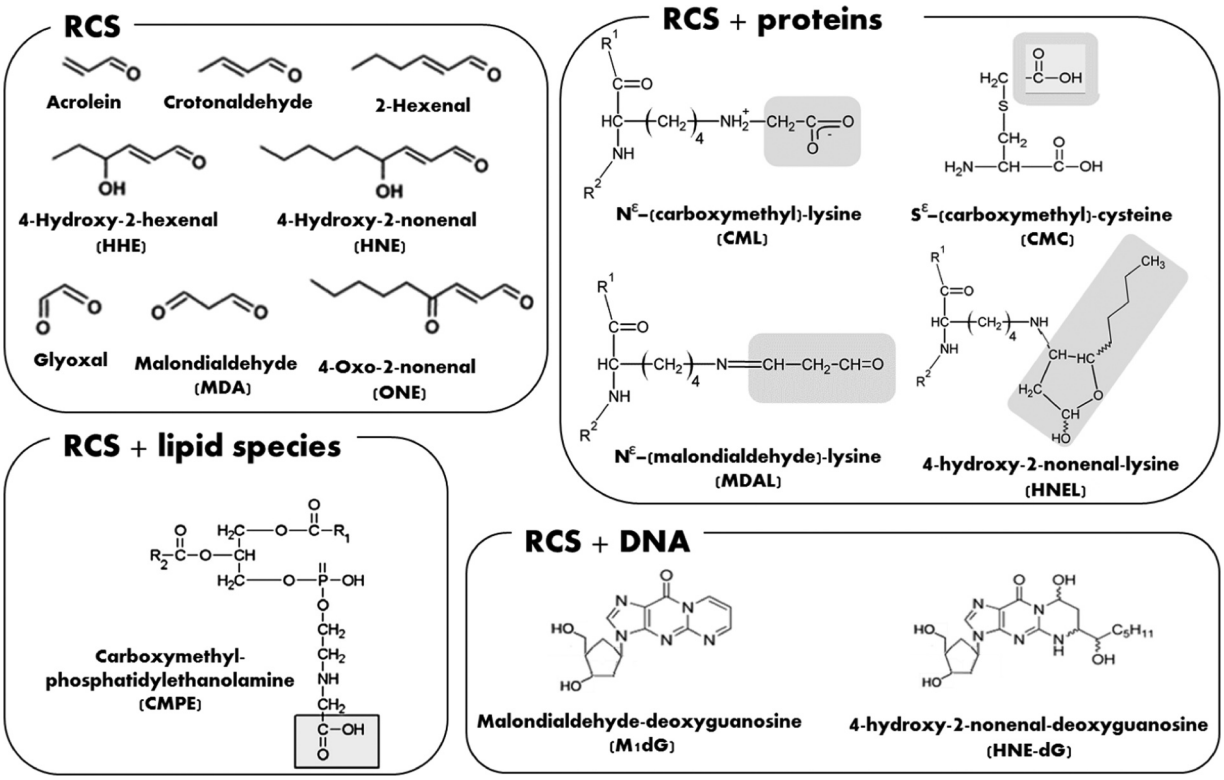

Fig. 2. General structures of principal reactive carbonyl species (RCS) detected in human brain. RCSs can react with nucleophilic groups in macromolecules (proteins, DNA and lipid species–aminophospholipids) resulting in their chemical, non-enzymatic and irreversible modification leading to the formation of a variety of adducts and crosslinks collectively called Advanced Lipoxidation End-products (ALEs).

transferase (GST) specifically designed to detoxify reactive carbonyl compounds [42,43], and GPx4 (phospholipid hydroperoxide glutathione peroxidase) designed to restore reduced states of membrane fatty acids from phospholipids to ensure membrane lipid homeostasis [44–47].

2.2. Cross-regional differences in vulnerability to lipoxidative damage

The high concentration of PUFAs in neuronal and glial cell membrane Gpl not only makes them prime targets for lipoperoxidative damage but also enables them to participate in long free radical chain reactions. Thus, from a given membrane fatty acid profile it is possible to calculate its peroxidizability index (PI) by combining this composition with the relative susceptibility of individual fatty acids to peroxidation. So, PI is a measure of the relative susceptibility of a given membrane fatty acid composition to peroxidative damage. The higher the value of PI, the greater the susceptibility of the membrane bilayer to lipid peroxidation [20,21].

Available evidence comparing 12 brain regions (spinal cord, medulla oblongata, cerebellum, substantia nigra, thalamus, amygdala, striatum, entorhinal cortex, hippocampus, temporal cortex, occipital cortex, and frontal cortex) reveals the presence of cross-regional differences in the PI in the human central nervous system and, consequently, establishes a region-dependent vulnerability [19] (see Table 1). In order to learn whether these differences followed a specific pattern, the relationship between the PI and their distribution according to the main subdivisions of the embryonic vertebrate brain following the caudal-cranial axis (from spinal cord to cerebral cortex) was evaluated [19]. The results demonstrated that the higher (more cranial) the brain region, the greater the PI. However, a greater PI is not necessarily associated with a higher degree of molecular damage. In fact, the case is quite the reverse. Thus, the higher the PI of the analyzed regions, the lower the lipoxidation-derived protein damage. This dissociation is due to the lower mitochondrial stress in the superior brain regions, and better neuronal adaptive response mediated by the antioxidant response-signaling pathway Nrf2 [19].

The cross-regional vulnerability differences have been also evidenced using a redox proteomics approach. Thus, a recent study analyzed, by western blot, levels of protein adduction by lipid peroxidation (LP) end-products neuroketal (NKT) and malondialdehyde (MDA), as markers of protein lipoxidative damage, in twelve brain regions in normal middle-aged individuals [48]. The findings demonstrated that the degree of molecular damage is region-dependent. Reinforcing this observation, another work using a redox proteomic profiling of neuroketal-adducted proteins in three human brain regions also verified a differential regional vulnerability [49].

Together, available evidence confirm the existence of a region-dependent vulnerability and the presence of cross-regional differences to lipoxidative damage in healthy adult human brain.

3. Lipids and lipoxidative damage during human brain aging

3.1. Lipids and brain aging

The physiological aging process induces changes at all levels of the biological organization which are offset by allostatic adaptive response mechanisms geared to preserving the composition and function within homeostatic limits. The cell membrane is not an exception, and, consequently, the longer the optimal membrane lipid composition is maintained, the better the cell survival and function [50]. However, deterioration in structure and function of the adult human brain during aging is not uniform throughout the brain. Consequently, human brain aging must be considered as a heterogeneous process probably subject to region-specific (and even cell-specific) vulnerability. Yet, regional vulnerability is often ignored in the study of brain aging. Regional vulnerability refers to the particular sensitivity of a given region to damage by distinct noxious stimuli and to its specific capacity to respond with the appropriate defenses. In this framework, it is plausible to hypothesize that cross-regional differences in human brain lipid composition, in addition to differences in oxidative stress conditions, could be responsible for this vulnerability which, in turn, leads to different rates of aging.

Two early studies, from the 1950s and 1960s [51,52], both performed in whole brain samples, offered the first evidence that the membrane lipid composition of the human brain changes with aging. They found that the amount of total lipids decreased after the second decade of life. Subsequent studies confirmed the occurrence of age-related lipid alterations in different areas of human brain. Overall, the described changes are aligned to the progressive and deleterious character of the aging process, and demonstrate that concentrations of most lipids in the human brain decrease after the age of 50. Nevertheless, the brain levels of different Gpls like PI, PE, and PC decrease very slowly with age, with less than 10% loss in the period between 40 and 100 years of age [53]. In another study, 10–20% loss of Gpl in different brain regions (gray matter, white matter, nucleus caudatus, hippocampus, pons, cerebellum, and medulla oblongata) was observed only in 90-year-olds compared to 35-year-olds, while phospholipid composition in various brain regions remained unchanged during aging [54]. In a similar way, another work found that the Gpl decrease starts slowly after age 20, and after age 80 becomes more pronounced, with no significant difference in lipid profile between male and female brains [55,56]. It has been also described how PE(P-) brain levels decrease 18% (till the age of 70) and 29% (till the age of 100), and SM brain levels decrease 12% and 20% in the same time period, respectively [57]. The effect of region-specific aging on brain cholesterol content has been also described. Thus, the studies of Svennerholm et al. [56] and Söderberg et al. [54] reported a reduction in cholesterol levels starting at 20 years of age in human frontal and temporal cortices, as well as hippocampus, nucleus caudatus, medulla oblongata, and cerebellum.

More recent studies focused on the mitochondrial and microsomal lipidome of frontal cortex, hippocampus, and entorhinal cortex of subjects from 20 to 100 years old [15–17], showing that minor lipid molecular species of Gpl from PC, PE, and PS classes and containing adrenic acid (22:4n-6) and AA specifically decreased along adult life in the three regions of the cerebral cortex, whereas particular PC, PE, and PS containing DHA increased or remained the same during the same period. Similarly, minor changes or none at all were observed in the distribution of lipid classes and fatty acid composition of lipid rafts from normal human frontal cortex throughout the human lifespan (24–85 years) [58].

An additional research line comes from studies focused on the analysis of the fatty acid compositional profile in different regions of the human cerebral cortex such as orbitofrontal cortex (OFC, Brodmann area 10) [59], prefrontal cortex [15], frontal cortex (area 8) [60], entorhinal cortex [17], and hippocampus [16], in healthy subjects ranging from 20 to 80 years old. No data are available from other regions of the human central nervous system. The findings of these studies point to some basic ideas: i) a general sustained and preserved fatty acid profile throughout the adult lifespan in most studied regions; ii) maintenance or minor changes with age in the saturated and monounsaturated fatty acid content; iii) decrease in the PUFA content from series n-6 with age, particularly affecting 20:4n-6 and 22:4n-6; and iv) maintenance or minor increase in 22:6n-3 content during aging with eventual reduction at a very advanced age.

Sustained SFA, MUFA, and DHA content with age could be interpreted as an adaptive response to aging to preserve neurons and cerebral function by helping to maintain the geometric properties of lipids and, consequently, functional properties such as exocytosis and membrane domain formation [5]. The meaning of changes (though minor) in the average chain length and double bond index at advanced ages, which can also influence the geometric properties of lipids and their functional properties, remains to be explored. In contrast to these

observations, decreases in PUFAn-6, and particularly the 20:4n-6 and 22:4n-6 fatty acid content, could have biological effects in that they are substrates for lipid mediators. Thus, one explanation for this finding lies in the reported decline in the PUFA biosynthesis pathway [60]; but there is also increased consumption by enzymes involved in anti-inflammatory pathways which synthetize a diversity of compounds with neuroprotective properties to ensure cell survival and functioning during normal aging [60]. For this reason it is proposed that during normal human brain aging, the lipid profile is particularly resistant to changes with age because they are under strict control to ensure neuronal survival and function.

Overall, the available evidence suggests that major adult human brain lipids undergo slight but progressive and significant changes in their concentrations and distribution during the aging process. However, the individual contribution of these lipid patterns to the aging process is as yet unknown. Therefore, a goal of future research is to define which types of lipids change with age in the different human brain areas and how they relate both to the function of the area and to the dysfunction leading to neuropathology. Indeed, it is not yet known whether the changes in the confirmed lipids represent neutral changes with age, changes causing physiological aspects of aging, or rather are beneficial responses to damaging agents. In any case, the described findings suggest that lipid species and their metabolism are closely linked to human brain aging.

3.2. Cross-regional differences in lipoxidative damage during human brain aging

Although the membrane lipid composition is largely maintained to guarantee cell survival and function, there is a continuous physiological lipoperoxidative attack on the membrane, and this membrane becomes a source of carbonyl compounds with the ability to damage other cellular components. Consequently, it is suggested that progressive lipoxidation-derived molecular damage is a conserved, central mechanism of age-related functional decline in human brain.

Thus, recently, the first study analyzing selective and specific protein damage markers by mass spectrometry in frontal cortex from healthy human covering a range of age between 43 and 86 years old was published [61]. The results demonstrated that there is an increase in the steady-state level of protein oxidative and lipoxidative damage in human frontal cortex over the adult lifespan, with a breakpoint at 60 years of age (the detected and quantified markers for protein oxidation were glutamic semialdehyde and aminoadipic semialdehyde; and carboxymethyl-lysine (CML) and carboxyethyl-lysine (CEL), for protein lipoxidation). This increase seems to be selective because temporal trajectories for the steady-state level of markers derived from the non-enzymatic modification of cysteine residues (carboxymethyl-cysteine, CMC; and succinyl-cysteine, SC) remained unchanged with age. This dissociation in the behavior of the different protein damage markers analyzed may be ascribed to the cellular homeostatic mechanisms behind their formation. Thus, the increase in the oxidation markers may reflect an increase in the net flux of free radical generation, but also a decrease in the activity of protein turnover mechanisms; and for protein lipoxidation, the increased levels can express an increased oxidative stress status (again free radical generation and protein turnover), but also an increased level in the content of PUFAs which act as substrate for the formation of RCS and these markers. In contrast, the unchanged content in markers derived from cysteine modification may be an indication of better preservation of cysteine residues and their functionality by specific repair mechanisms over the adult lifespan—mechanisms which are absent for other kind of markers. The presence of other kind of nonenzymatic modifications affecting cysteine residues cannot be discarded.

In another recent work [49] the regional differences in the protein damage markers derived from the RCS neuroketals (NKT) and malondialdehyde (MDA) in twelve brain regions (frontal cortex area 8, parietal cortex area 7, inferior temporal cortex area 20, occipital cortex 17–18, cingulate gyrus area 24, entorhinal cortex, hippocampus, head of the caudate, anterior putamen, thalamus, substantia nigra, and upper vermis) in two groups of individuals, middle-aged and old-aged, were analyzed. The middle-aged group presented no neuropathological lesions and the old-aged group presented neurofibrillary tangles (NFT) pathology at stage I-II considered consistent with normal brain aging [62,63]. Steady state levels of these markers were also analyzed as a function of age as continuous variables. The levels of NKT-modified proteins showed discrete regional variations in old-aged human brain. NKT-protein adducts were increased in frontal cortex, visual cortex, and substantia nigra, and reduced in thalamus when comparing the two groups. However, only NKT-protein adduct levels in the thalamus remained significantly decreased considering age as a continuous variable. In addition, reduced NKT adducts with age were identified in the putamen.

In contrast to NKT, MDA-lys showed wide regional presentation in aged human brain. Furthermore, this non-enzymatic modification affected more regions in old age when compared with NKT. Five brain regions display increased MDA protein adduction in old-aged individuals, including frontal cortex, parietal cortex, hippocampus, thalamus, and putamen. Curiously, decreased levels of MDA adduction with age occur in temporal cortex and entorhinal cortex when comparing middle-aged with old-aged individuals. However, only increased MDA protein adduction in frontal cortex, parietal cortex, and thalamus, and reduced MDA adduction in entorhinal cortex, remain significant when considering age as a continuous variable. Significantly reduced MDA adducts in the temporal cortex and increased adducts in the putamen were additionally recognized. Interestingly, in addition to MDA-lys, lipofuscin shows a nearly linear accumulation with age in the nervous system [64,65]. Whether regional lipofuscin deposition parallels regional accumulation of MDA adducts invites additional study.

So, the present findings show unexpectedly few variations restricted to certain brain regions and mainly involving the production of MDA adducts. It would be interesting to integrate trajectories of different variables like free radical production, changes in fatty acid profile, and lipoxidative damage to explain the differential impact of aging according to the brain region.

4. Selectivity of lipoxidation-derived molecular damage in human brain

4.1. Selective protein lipoxidation in human brain and during brain aging

Although the steady state levels of ALEs in proteins increase in human brain in a region-specific way during aging, such evidence does not elucidate the specific mechanisms that cause losses in particular cellular/tissue functions. Is there a selective pattern of lipoxidative protein damage? If true, what are the protein targets? Why this pool of proteins (if any) is modified? What is the putative mechanism by which selective protein damage plays a causal role in aging? Few studies have addressed these questions.

The human transcriptomics study demonstrates the existence of 20,344 putative protein-coding genes, with about 74% of these genes expressed in the brain compared to other human tissues [66]. More specifically, in total 10,465 protein entries (corresponding to 7797 protein-coding genes) have been detected and identified in healthy human brain by mass spectrometry-based proteomics [67]. Assuming this number of brain proteins, available evidence demonstrates that only 0.7% of the healthy adult brain proteome is lipoxidatively modified (71 proteins from 10,465) (see Table 2). Importantly, the degree of lipoxidative damage increases with age, and in all the identified proteins increased lipoxidation is not due to a higher content of the corresponding protein but rather to increased flux of protein damage. This small but significant pool of lipoxidized protein probably will increase with time because i) currently available studies are exclusively

Table 2
Lipoxidized proteins identified by redox proteomics from healthy adult/aged human cerebral cortex.

ID (entry human)	Protein	Gene	Main location	ALEs detected	Biological process	Reference(s)
P06733	alpha-Enolase	ENO1	Cytosol, cell membrane, nucleus	HNE, MDA, NKT	Energy metabolism (glycolysis)	[48,77–81,112]
P09104	gamma-Enolase	ENO2	Cytosol, cell membrane	Lipofuscin, MDA	Energy metabolism (glycolysis)	[38,112]
P04075	Fructose-bisphosphate aldolase A	ALDO A	Cytosol	HNE	Energy metabolism (glycolysis)	[77–81]
P04406	Glyceraldehyde − 3-phosphate dehydrogenase	GAPDH	Cytosol, cytoskeleton, nucleus	Lipofuscin, NKT	Energy metabolism (glycolysis)	[38,48]
P07195	Lactate dehydrogenase B chain	LDHB	Cytosol	HNE	Energy metabolism (glycolysis)	[77–81]
P00558	Phosphoglycerate kinase 1	PGK1	Cytosol	HNE	Energy metabolism (glycolysis)	[77–81]
P18669	Phosphoglycerate mutase 1	PGAM1	Cytosol, EVE, nucleus	NKT	Energy metabolism (glycolysis)	[48]
P14618	Pyruvate kinase	PKM	Cytosol, nucleus	HNE, NKT	Energy metabolism (glycolysis)	[48,77–81]
P60174	Triosephosphate isomerase	TPI1	Cytosol, EVE, nucleus	HNE, lipofuscin	Energy metabolism (glycolysis)	[38,77–81]
Q99798	Aconitate hydratase	ACO2	Mitochondrion	HNE, NKT	Energy metabolism (TCA cycle)	[48,77–81]
P00367	Glutamate dehydrogenase 1	GLUD1	Mitochondrion	MDA	Energy metabolism (TCA cycle)	[112]
P40926	Malate dehydrogenase	MDH2	Mitochondrion	HNE	Energy metabolism (TCA cycle)	[77–81]
P20674	Cytochrome c oxidase subunit 5a	COX5A	Mitochondrion	Lipofuscin	Energy metabolism (ETC)	[38]
P09622	Dihydrolipoyl dehydrogenase	DLD	Mitochondrion, nucleus	NKT	Energy metabolism (ETC)	[48]
O75489	NADH dehydrogenase (ubiquinone) iron-sulfur protein 3	NDUFS3	Mitochondrion	Lipofuscin	Energy metabolism (ETC)	[38]
P31930	Ubiquinol-cytochrome c reductase complex core protein 1	UQCRC1	Mitochondrion	MDA	Energy metabolism (ETC)	[112]
P25705	ATP synthase subunit alpha	ATP5F1A	Mitochondrion	HNE, lipofuscin, NKT	Energy metabolism (OxPhos)	[38,48,77–81]
P06576	ATP synthase subunit beta	ATP5F1B	Mitochondrion	Lipofuscin, MDA	Energy metabolism (OxPhos)	[38,112]
O75947	ATP synthase subunit d	ATP5H	Mitochondrion	Lipofuscin	Energy metabolism (OxPhos)	[38]
P48047	ATP synthase subunit o	ATP5PO	Mitochondrion	Lipofuscin	Energy metabolism (OxPhos)	[38]
P12277	Creatine kinase B-type	CKB	Cytosol	Lipofuscin, MDA, NKT	Energy metabolism (energy transduction)	[38,48,112]
P12532	Creatine Kinase U-type	CKMT1A	Mitochondrion	Lipofuscin	Energy metabolism (energy transduction)	[38]
P17174	Aspartate aminotransferase	GOT1	Cytosol	NKT	Neurotransmission	[48]
P80723	Brain acid soluble protein 1	BASP1	Cell membrane, growth cone	Lipofuscin, NKT	Neurotransmission	[38,48]
Q9UQM7	Calcium/calmodulin-dependent protein kinase type II subunit alpha	CAMK2A	Synapse, dendritic spine, dendrite	Lipofuscin	Neurotransmission	[38]
Q00610	Clathrin heavy chain 1	CLTC	Cytosol/vesicle/membrane	Lipofuscin	Neurotransmission	[38]
P09543	2',3'-cyclic nucleotide 3'-phosphodiesterase	CNP	Cytosol/EVE/membrane	Lipofuscin	Neurotransmission	[38]
Q16555	Dihydropyrimidinase-related protein 2	DPYSL2	Cytosol, cytoskeleton, membrane	HNE, lipofuscin, MDA, NKT	Neurotransmission	[38,48,77–81,112]
P15104	Glutamine synthetase	GLUL	Cytosol, mitochondrion	HNE, MDA	Neurotransmission	[77–81,112]
Q08722	Leukocyte surface antigen (CD antigen CD47)	CD47	Cell membrane	Lipofuscin	Neurotransmission	[38]
Q13449	Limbic system associated membrane protein	LSAMP	Cell membrane	Lipofuscin	Neurotransmission	[38]
P30086	Phosphatidylethanolamine-binding protein 1	PEBP1	Cytosol	HNE, NKT	Neurotransmission	[48,77–81]
P61981	14–3–3 protein gamma	YWHAG	Cytosol	Lipofuscin, NKT	Neurotransmission	[38,48]
P62258	14–3–3 protein epsilon	YWHAE	Cytosol/EVE	Lipofuscin	Neurotransmission	[38]
P63104	14–3–3 protein zeta/delta	YWHAZ	Cytosol/EVE	Lipofuscin	Neurotransmission	[38]
P17600	Synapsin − 1	SYN1	Golgi apparatus, synaptic vesicle	NKT	Neurotransmission	[48]
P60880	Synaptosomal-associated protein 25	SNAP25	Cell membrane, synaptosome	Lipofuscin	Neurotransmission	[38]
P61266	Syntaxin − 1B	STX1B	Membrane, cytoskeleton, nucleus	Lipofuscin	Neurotransmission	[38]
P61764	Syntaxin-binding protein 1	STXBP1	Cytosol, membrane	Lipofuscin	Neurotransmission	[38]
P63027	Vesicle-associated membrane protein 2	VAMP2	Cell membrane, synaptic vesicle, membrane	Lipofuscin	Neurotransmission	[38]
P60709	beta-Actin	ACTB	Cytosol (cytoskeleton)	HNE, Lipofuscin, MDA	Cytoskeleton	[38,77–81,112]
Q05193	Dynamin 1	DNM1	Cytosol (cytoskeleton)	Lipofuscin	Cytoskeleton	[38]
P14136	Glial fibrillary acidic protein	GFAP	Cytosol (cytoskeleton)	Lipofuscin, MDA, NKT	Cytoskeleton	[38,48,112]
P07196	Neurofilament light polypeptide	NEFL	Cytosol (cytoskeleton)	Lipofuscin, MDA, NKT	Cytoskeleton	[38,48,112]
P07197	Neurofilament medium polypeptide	NEFM	Cytosol (cytoskeleton)	NKT	Cytoskeleton	[48]
Q13813	Spectrin alpha chain brain	SPTAN1	Cytosol (cytoskeleton)	Lipofuscin	Cytoskeleton	[38]
Q01082	Spectrin beta chain, brain 1	SPTBN1	Cytosol (cytoskeleton)	Lipofuscin	Cytoskeleton	[38]
P04216	Thy − 1 membrane glycoprotein	THY1	Cell membrane	Lipofuscin	Cytoskeleton	[38]
P68363	Tubulin alpha 1B chain	TUBA1B	Cytosol (cytoskeleton)	HNE, Lipofuscin, MDA	Cytoskeleton	[38,77–81,112]
P07437	Tubulin beta chain	TUBB	Cytosol (cytoskeleton)	Lipofuscin, MDA	Cytoskeleton	[38,112]

(*continued on next page*)

Table 2 (*continued*)

ID (entry human)	Protein	Gene	Main location	ALEs detected	Biological process	Reference(s)
O94811	Tubulin polymerization-promoting protein (TPPP)	TPPP	Cytosol (cytoskeleton), nucleus	Lipofuscin, NKT	Cytoskeleton	[38,48]
P08670	Vimentin	VIM	Cytosol (cytoskeleton), nucleus	MDA	Cytoskeleton	[112]
P02511	alpha-Crystallin B chain (Heat Shock Protein B5)	CRYAB	Cytosol, nucleus	Lipofuscin, NKT	Proteostasis	[38,48]
P49411	Elongation factor Tu	TUFM	Mitochondrion	HNE	Proteostasis	[77–81]
Q9BY44	Eukaryotic translation initiation factor 2A	EIF2A	Cytosol	HNE	Proteostasis	[77–81]
P10809	Heat shock protein 60KDa	HSPD1	Mitochondrion	Lipofuscin, MDA, NKT	Proteostasis	[38,48,112]
P0DMV8	Heat shock protein 70KDa protein 1A	HSPA1A	Cytosol, nucleus, cytoskeleton	HNE	Proteostasis	[77–81]
Q99497	Protein/nucleic acid deglycase DJ−1	PARK7	Cytosol, nucleus, mitochondrion, cell membrane	NKT	Proteostasis	[48]
P09936	Ubiquitin carboxyl-terminal hydrolase L1	UCHL1	Cytosol, endoplasmic reticulum	Lipofuscin, NKT	Proteostasis	[38,48]
P16152	Carbonyl reductase (NADPH) 1	CBR1	Cytosol	HNE	Antioxidants	[77–81]
P09601	Hemeoxygenase 1	HMOX1	Endoplasmic reticulum	HNE	Antioxidants	[77–81]
P04179	Manganese superoxide dismutase	SOD2	Mitochondrion	HNE	Antioxidants	[77–81]
P30041	Peroxiredoxin 6	PRDX6	Cytosol, lysosome	HNE	Antioxidants	[77–81]
P00915	Carbonic anhydrase 1	CA1	Cytosol	NKT	O_2/CO_2/heme metabolism	[48]
P69905	Hemoglobin subunit alpha	HBA1	Cytosol, EVE	NKT	O_2/CO_2/heme metabolism	[48]
P30043	NADPH-Flavin reductase	BLVRB	Cytosol	NKT	O_2/CO_2/heme metabolism	[48]
P13637	Sodium/potassium-transporting ATPase subunit alpha−3	ATP1A3	Cell membrane	Lipofuscin	Ion transport	[38]
P21796	Voltage-dependent anion-selective channel protein 1	VDAC1	Mitochondrion, cell membrane	Lipofuscin	Ion channel	[38]
P36543	V-type proton ATPase subunit E1	ATP6V1E1	Cytosol, endosome, EVE, lysosome, plasma membrane	Lipofuscin	Ion transport	[38]
B1AKQ8	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta1	GNB1	Not described	Lipofuscin, MDA	Signal transduction	[38,112]
P09471	Guanine nucleotide binding protein G(O) subunit alpha	GNAO1	Cell membrane	Lipofuscin	Signal transduction	[38]

Gene, main location, and biological process are based on what was reported in the UniProt database (http://www.uniprot.org/). EVE, extracellular vesicular exosome. Abbreviations: TCA cycle, tricarboxylic acid cycle; ETC, electron transport chain; OxPhos, oxidative phosphorylation; ALEs, advanced lipoxidation end-products; NKT, neuroketals; MDA, Malondialdehyde; HNE, hydroxynonenal. Sources: [38,48,77–81,112].

restricted to different areas of the human cerebral cortex (e.g. parietal cortex, frontal cortex, temporal cortex, entorhinal cortex, cingulate gyrus, and hippocampus), and ii) the ALEs detected are limited to those derived from HNE, lipofuscin, MDA, and NKTs. Consequently, further studies extending analyzed brain regions and the lipoxidation markers detected are needed to obtain a more detailed view.

The potential selectivity of lipoxidation and its derived molecular damage is supported by several works (some articles/reviews addressing the topic of the selectivity of lipoxidation in different experimental models are [68–71]. In proteomic studies, frequently only a subset of proteins is found to get modified in a complex system, and only one or a few residues become adducted in a given protein, generally, residues with particularly low pKa or high reactivity. Also the structure of the modifying species influences the selectivity of the modification.

The structural characterization of the modified proteins in the healthy adult human brain with PredictProtein software analysis (https://www.predictprotein.org/) reveals some shared specific traits which may explain this specificity rendering proteins more susceptible to oxidative damage [48]. Thus, i) the predominant structures of lipoxidized proteins are alpha helix and loops, ii) most proteins (excepting cytoskeletal proteins) are globular and form soluble coiled-shaped molecules with hydrophobic groups at the core and exposed hydrophilic groups to the medium, and iii) among the most recurrent amino acids encountered in the exposed regions is lysine, in addition to glutamic acid and aspartic acid, a frequent target of non-enzymatic modification on proteins.

Another factor that may be important in the selectivity of modified proteins is the spatial location. Localization of proteins may make them more vulnerable as a result of their environmental conditions. Positioning refers to a particular cell type or to a subcellular compartment. From data in Table 2, an example of cell type-related vulnerability is GFAP localized in astrocytes which are brain producers of DHA and its lipid peroxidation derivatives. Regarding subcellular localization, several lipoxidized proteins are located in the mitochondria, the principal source of damaging free radicals; other proteins are located in membranes belonging to different subcellular compartments or even cell membranes, which show a high content in PUFAs. In contrast, other damaged proteins are those of the cytoskeleton, or they are located in the growth cones, involved in neurotransmission, or present in synaptic vesicles. It is worth stressing that axons and synaptic terminals have high bioenergetic demands achieved by continuous mitochondrial activity and recruitment, and rapid energy transduction. In addition, axons are radial structures with a small diameter in comparison with the cell body which heightens the probability of stochastic interactions between free radicals and membrane lipids, thus propitiating lipid peroxidation and further non-enzymatic modification of proteins.

Overall, these observations show that i) the cerebral cortex in healthy adults contains lipoxidized proteins, the levels of which vary among regions, ii) the ALE-modified proteins increase in aged subjects, and iii) the selectivity of molecular damage is associated with specific structural traits and spatial location.

4.2. Functional clustering of lipoxidatively-damaged proteins

The identification of specific lipoxidation-modified proteins in the brain of adult and aged subjects provides an overview of the selective cellular functions that are affected during the aging process. The proteins listed in Table 2 show us the biological processes affected by RCS-related protein damage. If we consider these molecular pathways, we verify that energy metabolism (with 31% of modified proteins, n = 22), neurotransmission (including neuronal communication, synaptic plasticity, and other processes) (with 25% of modified proteins, n = 18), and cytoskeleton (17% of modified proteins, n = 12) are the main affected cellular functions. Other significant molecular pathways involved but with a lesser number of modified proteins are proteostasis (9.8%, n = 7 modified proteins), antioxidants (5.6%, n = 4), O_2/CO_2/heme metabolism (4.2%, N03), transport (4.2%, n = 3), and signal transduction (2.8%, n = 2).

There is an extensive evidence that energy metabolism is particularly affected during aging [8,72–74]. The reported observations give some clues about the molecular substrates of energy failure with aging after the identification of key proteins as targets of lipoxidative damage including proteins of glycolysis ENO1, ENO2, ALDO A, GAPDH, LDHB, PGK1, PGAM1, PKM, and TPI1; proteins of the tricarboxylic acid (TCA) cycle ACO2, GLUD1, and MDH2; protein subunits of the mitochondrial electron transport chain complexes COX5A, DLD, NDUFS3, and UQCRC1; and different protein subunits of mitochondrial ATP synthase or complex V responsible for oxidative phosphorylation. All of these proteins are components of coupled processes which are necessary to fulfill the ATP requirements of cells. Neuronal activity is highly dependent on these processes since, in normal conditions, glucose is the exclusive energy substrate for the brain [75]. In addition, CKB and CKMT1A are in charge of rapid ATP production from phosphocreatine reservoirs in response to acute increased energy demands in neurons, but both proteins are also key players in the 'phosphocreatine circuit' for cellular energy homeostasis [76]. Thus CKB and CKMT1A provide neurons with a reservoir and also an alternative source of ATP to glycolysis, TCA cycle, and respiration.

The second major biological process that is affected during aging based on the number of lipoxidized proteins is neurotransmission [38,48,77–81]. The 18 affected proteins are: GOT1, BASP1, CAMK2A, CLTC, CNP, DPYSL2, GLUL, CD47, LSAMP, PEBP1, YWHAG, YWHAE, YWHAZ, SYN1, SNAP25, STX1B, STXBP1, and VAMP2. These proteins are involved in several functions such as neurotransmitter metabolism, axon growth and guidance, growth cone collapse and cell migration, neuron differentiation, synaptic transmission and plasticity, synaptic vesicle trafficking, signaling pathways, and regulation transcriptional activity, among others. These modifications together with those linked to the high energy demands make synapses especially vulnerable to oxidative stress damage. Interestingly, these biological processes potentially affected by the modified proteins are aligned with the concept that most of the functional decline associated with normal brain aging is caused by relatively subtle changes such as changes in the molecular profile of synapses, altered spine morphologies, reductions in spine densities, or loss of dendrites [7,82].

Cytoskeletal proteins are also lipoxidized being the third major biological process with a great number of modified proteins (n = 12). The affected proteins are: beta-Actin, dynamin 1, glial fibrillary acidic protein, neurofilament light polypeptide, neurofilament medium polypeptide, spectrin alpha chain brain, spectrin beta chain brain 1, thy-1 membrane glycoprotein, tubulin alpha 1B chain, tubulin beta chain, tubulin polymerization-promoting protein (TPPP), and vimentin. Thus, proteins belonging to the main components of cytoskeleton seem to be affected: microfilaments, intermediate filaments, and microtubules. These findings are in line with previous observations showing that neurofilaments are major targets of HNE adduction in mice nervous system [83,84]. Consequently, general neuronal and glial processes linked to cytoskeleton such as the cytoskeleton organization itself, cytoskeleton-dependent intracellular transport, and maintenance of the integrity of the microtubule network and microtubule-based processes can become dysfunctional as a consequence of the non-enzymatic modification of their protein components. But additional functions more specifically linked to neural cells which require cytoskeleton integrity can also be affected secondarily to protein lipoxidation [7,82]. Among these functions are vesicle trafficking, synaptic plasticity, axon guidance, development of the central nervous system, maintenance of the neuronal caliber, and synaptogenesis.

Seven damaged proteins are involved in proteostasis. These proteins are: alpha-crystallin B chain (heat shock protein B5) (CRYAB), elongation factor Tu (TUFM), eukaryotic translation initiation factor 2A (EIF2A), heat shock protein 60KDa (HSPD1), heat shock protein 70KDa protein 1A (HSPA1A), protein/nucleic acid deglycase DJ-1 (PARK7),

and ubiquitin carboxyl-terminal hydrolase L1 (UCHL1). Whereas TUFM and EIF2A are involved in protein biosynthesis and HSPD1 participates in mitochondrial protein import and macromolecular assembly, folding of proteins, and apoptotic process, the rest of the proteins (UCHL1, CRYAB, HSPA1A, and PARK7) play a relevant role in oxidative stress homeostasis. Thus, UCHL1 participates in processing of ubiquitin precursors and ubiquitinated proteins for proteasomal degradation [85], and CRYAB shows chaperone-like activity and prevents aggregation of proteins under stress conditions [86]. HSPA1A is a molecular chaperone involved in the protection of the proteome from stress, playing a pivotal role in the protein quality control system, ensuring the correct folding of proteins and the re-folding of misfolded proteins, and controlling the targeting of proteins for subsequent degradation [87–89]. PARK7 is a protein and nucleotide deglycase that catalyzes the degradation of adducts formed between amino groups of proteins or nucleotides and RCS. Thus, it functions as a protein repair system [90–93]. It deglycates cysteine, arginine, and lysine residues in proteins, and thus reactivates these proteins by reversing non-enzymatic modification by RCS. Analogously, it also functions as a nucleotide deglycase able to repair modified guanine in the free nucleotide pool (GTP, GDP, GMP, dGTP) and in DNA and RNA. Furthermore, it plays an important role in cell protection against oxidative stress and cell death, acting as an oxidative stress sensor and redox-sensitive chaperone and protease. And finally, it is involved in neuroprotective mechanisms linked to mitochondrial uncoupling proteins, L-type channels, and inflammatory responses. So the modified chaperones are deeply involved in maintaining neuronal oxidative stress homeostasis. Assuming that nonenzymatic modifications are mostly linked to the loss of function, it may be proposed that the inactivation of the modified chaperones [85,92,94,95] are involved in brain aging by promoting cellular oxidative stress.

Reinforcing the dysfunction in oxidative stress homeostasis derived from the modified chaperones, lipoxidation-derived damage also affects some antioxidant enzymes such as carbonyl reductase (NADPH)1 (CBR1), hemeoxygenase 1 (HMOX1), manganese superoxide dismutase (SOD2), and peroxiredoxin 6 (PRDX6). CBR1 participates in the reduction of carbonyl compounds; HMOX1 has cytoprotective effects since an excess of free heme sensitizes cells to undergo apoptosis; SOD2 destroys superoxide radicals generated by mitochondrial complex I and III (the main cell generators of free radicals), and PEDX6 has antioxidant properties.

Three proteins related to heme metabolism and O_2 and CO_2 regulation, biliverdin reductase B (BLVRB), carbonic anhydrase (CA), and hemoglobin 1 A (HB1A), are also lipoxidatively damaged. CA participates in the conversion of CO_2 into bicarbonate and participates in the transport of CO_2 out of the tissues. BLVDR regulates the final step in heme metabolism, but it also regulates glucose metabolism and has neuroprotective effects [96]. HBA1 is a component of hemoglobin, the role of which in the nervous system is still poorly understood. Hemoglobin has been found in neurons where it probably plays a role in O_2 transport or in the regulation of cytosolic neuronal O_2 [97]. Lipoxidative damage to these three proteins tags cell O_2 and CO_2 regulation as a target of putative cellular respiratory dysfunction in the elderly.

Finally, molecular interactions of lipoxidized proteins in the human cerebral cortex were explored to identify systems that may be indirectly impaired as a result of primary protein lipoxidation and presumed loss of function. Ubiquitin C has been identified as the central node of the network of interactions [48]. Ubiquitin conjugation to proteins plays a cardinal role in the ubiquitin/proteasome system and protein turnover which has been identified as being affected by aging [98,99]. Since ubiquitin conjugation to target proteins occurs through the ε-amino group of lysine, RCS adduction of this group can restrain protein-ubiquitin interactions and then hamper the degradation of altered proteins. In addition, 50% of the ubiquitin binding sites are not involved in protein degradation by the ubiquitin/proteasome system [100], thus suggesting that ubiquitin serves in other molecular pathways. If true, several pathways can be deregulated as a result of altered ubiquitin conjugation with lipoxidized proteins [101].

5. ATP-synthase is a key lipoxidative target in human brain aging

5.1. Human ATP-synthase structure and function

Mitochondrial dysfunction and energy metabolism deficiencies have been recognized as molecular events in human brain aging and have been correlated with impairments of cognitive abilities. ATP synthase is a macromolecular structure located in the inner mitochondrial membrane and is the last complex (complex V) of the electron transport chain to play a key role in energy metabolism. Complex V has a central role in cellular energy (as ATP) supply.

Human mitochondrial ATP synthase is a complex of 583 kDa. It is composed of 15 different protein subunits and organized into two domains: a membrane-extrinsic and matrix-oriented F1 (371 kDa) catalytic domain, and a membrane-embedded F0 (212 kDa) domain, the two connected by a peripheral and central stalk [102–106]. More specifically, the F0 domain is a trans-membrane channel that translocates protons and F1, a synthase domain that binds to ADP and inorganic phosphate and synthesizes ATP on its surface. The coupling between F0 and F1 and coordination of conformational changes of their subunits is critical for efficient ATP generation.

The F1 domain is composed of 5 subunits with a stoichiometry of $\alpha 3\beta 3\gamma\delta\varepsilon$; the component $(\alpha\beta)3$ is the catalytic head and fundamental structure into which the $\gamma\delta\varepsilon$ central stalk rotor penetrates. The F0 domain consists of 10 subunits: a, b, c, d, e, f, g, OSCP (oligomycin sensitivity conferring protein), F6, and A6L. These subunits shape a ring that shuttles protons across the membrane, forms the peripheral stalk connecting the catalytic head to the membrane stator, and participates in the formation of ATP synthase dimers [107] that self-assemble in longer ribbons important for cristae formation [108,109].

F0F1 ATP synthase activity is regulated by several factors such as membrane electrochemical potential, allostery, including ATPase inhibitory peptides, and protein synthesis [102], as well as membrane lipid environment [110], and post-translational modifications [111]. Interestingly, some of the functional properties of ATP synthase and regulatory mechanisms are linked to the integrity of specific residues such as lysine, arginine, and cysteine [102,110,111]. Thus, for instance, specific post-translational and reversible modifications like acetylation and methylation play important roles in ATP synthase regulation by modifying ε-amino groups of lysine, with the resulting conformational changes of the active sites and decreased enzymatic activity [111]. In a similar way, the integrity of structural lysine residues is a key point to promote the interaction with membrane lipids, particularly cardiolipin, in order to ensure correct ATP synthase activity [110].

5.2. ATP-synthase as target of lipoxidative damage in human brain aging

Available evidence demonstrates that ATP synthase is a systematically damaged key protein. The lipoxidation-derived protein adducts described are formed from hydroxynonenal, malondialdehyde, neuroketals, or lipofuscin (see Table 2). Lipoxidation damage affects preferentially, but not exclusively, to subunits α and β (see Table 2); although the specific residues targeted are still unknown. The immunohistochemical studies suggest that the only cell type damaged are neurons [80]; modified ATP synthase has been detected in different regions of the human cerebral cortex, and this lipoxidation-derived protein damage increases in the elderly [48,80,112].

This preferential and selective non-enzymatic modification of ATP synthase is related to several factors such as structural traits, location, and functional characteristics that can influence this specificity (see Fig. 3). Concerning structural traits, it has been demonstrated that the presence of alpha helices and loops, globular shapes that additionally form soluble coiled-shaped molecules with hydrophobic groups to the

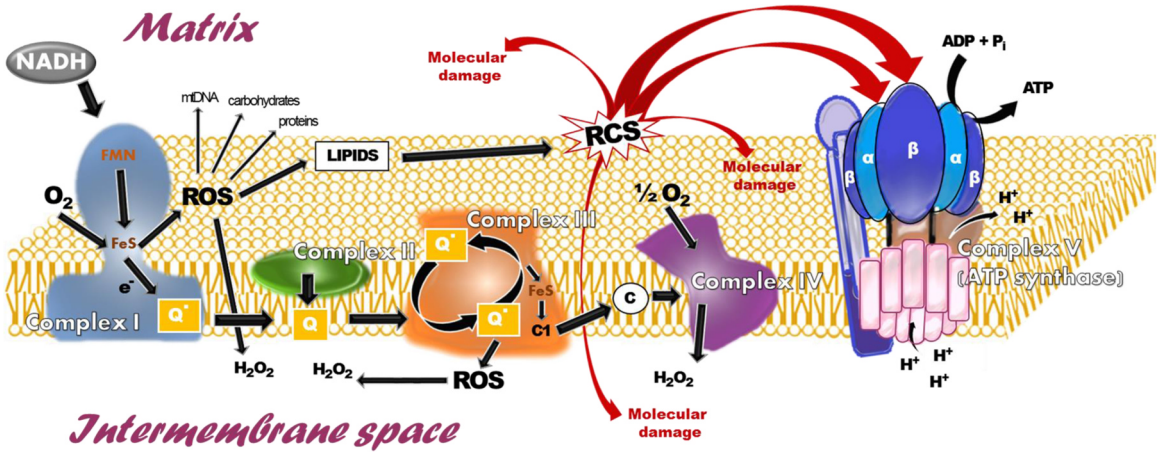

Fig. 3. Schematic diagram of mitochondrial processes that are important for aging. It shows that mitochondrial complex I and complex III are the main free radical (ROS) generators. Oxygen radicals attack lipids, carbohydrates, proteins, and DNA. The products of lipid peroxidation include highly reactive molecules (RCS, reactive carbonyl species) that can cause lipoxidative damage to mitochondrial DNA, proteins, and aminophospholipids and, by extension, damage to other cellular components. Mitochondrial ATP-synthase is a key lipoxidative target in human brain aging.

center and exposed hydrophilic groups, and the presence and exposure of amino acids like lysine which are particularly vulnerable, render ATP synthase susceptible to lipoxidative damage [48]. As to location, ATP synthase is a mitochondrial complex located inside the inner membrane and oriented toward the mitochondrial matrix. Thus, ATP synthase is exposed to a potentially dangerous environment because of proximity to the main generators of free radicals (mitochondrial complex I and III) at the cellular level, and its insertion in a lipid bilayer highly enriched in PUFAs.

The non-enzymatic modification of the specific subunits α and β probably changes ATP-synthase conformation, leading to the inactivation of the complex and defects in ATP synthesis. It has been demonstrated that ATP synthase activity is decreased in the cerebral cortex of aged individuals [80]. Importantly, ATP synthase lipoxidation and its loss of activity are not accompanied by reduced total expression levels of this protein [80]. In fact, it is documented that ATP synthase content does not change with age [61]. Therefore, the non-enzymatic and irreversible modification by carbonyl compounds derived from lipid peroxidation of ATP synthase likely explains their reduced activity and reduced ATP levels during the human brain aging process. Furthermore, this loss of ATP synthase activity can affect the electron transport chain, inducing a mitochondrial dysfunction leading to increased reactive oxygen species production, and consequently impairment, in cellular oxidative stress conditions, with the subsequent neuronal damage.

6. Conclusions

Overall, the available evidence provides robust information about increased steady state levels of protein damaged by lipoxidation reactions with aging in a region-specific way which may compromise vital cell functions such as energy metabolism, neurotransmission, cytoskeleton, proteostasis, antioxidants, and O_2/ CO_2/heme homeostasis. Post-translationally modified proteins resulting from lipoxidative damage may be considered putative collaborative factors contributing to neuronal aging. Protein vulnerability to non-enzymatic modifications is related to the particular structural traits and spatial location at the subcellular level. Mitochondrial ATP synthase is a key lipoxidative target in human brain aging. Importantly, since lipoxidative damage to proteins is already identified in middle-aged normal individuals and increases physiologically in the elderly, it seems reasonable to act upon the appropriate free radical-producing targets and lipid metabolism at the appropriate middle-age window.

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Author contributions

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Notes

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